

## Reproduction in Free-Ranging Male *Propithecus verreauxi*: The Hormonal Correlates of Mating and Aggression

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reproductive behavior

**ABSTRACT** Endocrine studies of captive strepsirrhine primates suggest that physical environment and social factors mediate inter-individual variations in testicular function and serum testosterone (sT) in males. While these studies have made major contributions to our understanding of the individual proximate mechanisms influencing androgen activity in male strepsirrhines, none have investigated how these mechanisms work coincidentally in free-ranging populations. In this study we used fecal steroid analysis to examine androgen-behavior interactions associated with reproduction in free-ranging male *Propithecus verreauxi*. Behavioral and hormone data were collected from two social groups during the 1990–91 and 1991–92 breeding seasons at Beza Mahafaly, Madagascar. Solid phase and radioimmunoassay techniques were used to quantify testosterone (T) in 105 desiccated fecal samples collected weekly from seven males. Results suggest that 1) solid phase extraction and radioimmunoassay techniques were reliable and accurate methods for quantifying T in sifaka feces; 2) fecal T (fT) elevations spanned a minimum of 4 months, peak levels occurring 1 month prior to the January onset of the breeding season; 3) fecal T concentrations were influenced by developmental factors and, among mature males, social factors associated with rank, intergroup aggression, and group instability. *Am J Phys Anthropol* 105:137–151, 1998. © 1998 Wiley-Liss, Inc.

Laboratory studies of the socioendocrinology of reproduction in strepsirrhine primates suggest that physical environment and social factors mediate variations in testicular function and serum testosterone (sT) levels in males (reviewed in Izard, 1990; Perret, 1992). Photoperiod (Petter-Rousseaux, 1970), social group composition (Perret, 1977, 1992), social dominance (Schilling et al., 1984; Perret, 1992), and intrasexual competition during the breeding season (Foerg, 1982) have all been shown to influence male reproductive function. While these studies have advanced our understanding of how a number

of proximate mechanisms influence gonadal activity in captive strepsirrhines, none have examined how these mechanisms interact to

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influence gonadal function in free-ranging populations. A component of a broader study of reproduction and mating in *P. verreauxi*, this study used radioimmunoassays (RIA) of fecal testosterone (fT) to examine the developmental and social factors affecting testicular function in free-ranging sifaka at Beza Mahafaly Special Reserve, Madagascar.

This paper reports the results of a study of the hormonal correlates of reproduction in free-ranging male *Propithecus verreauxi*, using fT as an index of reproductive function. Although recent data suggest that fecal steroid analysis can reliably assess ovarian function in both captive (Perez et al., 1988; Shideler et al., 1994) and free-ranging female (Strier and Ziegler, 1994; Stavisky et al., 1995) primates including sifaka (Brockman et al., 1995; Brockman and Whitten, 1996), similar hormonal data are lacking for males. The validity of RIA techniques in sifaka fecal extracts was examined in tests of parallelism, accuracy, and reliability. The behavioral significance of fT was tested by comparing composite and individual fT profiles to chronological and behavioral events that have been reported to reflect sT.

Verreaux's sifaka are diurnal, seasonally reproducing strepsirrhine primates inhabiting riverine and dry forests of south and southwest Madagascar (Tattersall, 1982). Social groups at this site range in size from two to 13 individuals in which females are dominant to males (Richard, 1987; Richard and Nicoll, 1987; Richard et al., 1991). Females exhibit a minimum 3 month breeding season beginning in January, during which most females are receptive to males for 1 to 4 days once during the 3-month period. Behavioral estrous is asynchronous within and between groups (Brockman and Whitten, 1996). Males and females typically mate with multiple partners in their own and neighboring groups. Mating is limited by intrasexual competition associated with male guarding and copulatory harassment by males and females (Brockman, 1994); it is enhanced through visits and increased encounters with neighboring groups (Richard, 1974, 1992; Brockman, 1994). The developmental factors affecting testicular function in male sifaka are predicted to reveal age-specific variations in T excretion, adult males

exhibiting a significantly higher level of fT than that observed in subadult and juveniles. We also expect that while fT levels may be somewhat elevated during mating, higher levels should be more strongly associated with rank, intergroup aggressive encounters, and group instability.

The objectives of this study were to: 1) validate (RIA) methods for assessing testosterone in fecal extracts of male sifaka, 2) examine levels and temporal patterning of fT excretion in males during the breeding season, and 3) elucidate potential developmental and social factors mediating T excretion and male reproductive behavior.

## MATERIALS AND METHODS

### Subjects and study period

Data were collected from the Vaovao (VV) and Vavy Masiaka (VM) social groups at Beza Mahafaly during the 1990–91 and 1991–92 breeding seasons. Data were obtained from December 10, 1990 through March 14, 1991 and December 5, 1991 through March 27, 1992 for the VV and VM males, respectively. Opportunistic demographic and hormonal data were also collected from six adult males during the August 1996 birth season to obtain preliminary estimates of non-breeding season levels of fT. During the 1990–91 field season, the modal group composition of the 28–30 social groups was three adult males and two adult females (Brockman, 1994). The study population was particularly conducive to reproductive studies because not only were most individuals collared and tagged, but they were well habituated and had detailed demographic and social group histories spanning the previous 7 years (Richard et al., 1991, 1993; Richard, 1992).

**Determination of age estimates.** Age estimates of the five marked males in this study were derived from birthdates, degree of toothwear (Richard, 1992; Richard et al., 1993; unpub. data), and assessments of relative testes size. Qualitative data on testes size were collected during the breeding season when testes are expected to reach their maximum size in this seasonally breeding primate. Age-related differences in testes size were visually assessed and photo-

graphed from interindividual comparative observations of testes at distances of 1 m or less. Age classes were assigned to the two unmarked males (UC and Fd) based upon body and testes size similarities to marked males.

**Age classes.** The males in this study were drawn from three age classes (Richard et al., 1991, 1993): *juvenile males* ( $n = 1$ ), which were small, exhibited no tooth wear, and had undescended or diminutive testes; *subadult males* ( $n = 2$ ), which had not acquired the body or testes size of adult males; and *adult males* ( $n = 4$ ), which were large and muscular, had moderate toothwear, and large testes. These age classes approximate chronological ages of 1–2 years, 4–5 years, and 6 years and over, respectively.

#### Collection and analysis of behavioral data

Continuous and ad libitum sampling methods were used to collect qualitative data on male reproductive behavior 6 to 7 days per week. Behavioral data obtained from the seven males were equally distributed over the two morning and three afternoon/evening sampling periods. Observations began at dawn (0600) and terminated at dusk (when the animals retired at 1800), and broke for 2 hours mid-day coincident with the animal's rest period. Exceptions to this regimen occurred when females were in estrus; observations at this time were recorded from 0600 to 2000. Behavioral data were analyzed to focus on differences in frequency of behavior over time. These data were then checked for normality and equal variance, and depending upon the results of these tests, parametric or nonparametric tests were used to examine differences and trends in the data. Significance was set at  $P < 0.05$ .

Reproductive behavior included all sexual and aggressive interactions exhibited during the breeding season, the latter being considered a form of mate competition (Richard, 1992). Sexual behaviors recorded were mating where intromission and thrusting were unambiguously observed, and mounts and mount attempts, the latter typically lasting 3 seconds or less and not associated with intromission and thrusting. Aggressive behaviors recorded included lunges, cuffs,

grabs, bites, chases and fights. Dominance was determined based on the consistent direction and outcome of aggressive behavior, dominant and subordinate males being the sole initiators/winners and recipients/losers of aggressive encounters, respectively (Sade, 1967; Hausfater, 1975). While perhaps less appropriate for describing the subtleties of male relationships outside the breeding season (Richard, 1992), the concept of dominance is crucial to an understanding of how hormone-behavior interactions are expressed in differently ranked males competing for sexual access to estrous females.

#### Collection and preservation of fecal samples

One hundred five weekly fecal samples were collected from seven males in the VV and VM social groups (Table 1). Fecal samples (1–15 g) were collected in their entirety immediately after voiding, then packaged, labeled, and desiccated using procedures described previously (Brockman, 1994; Brockman and Whitten, 1996). At the end of the field seasons the samples were shipped to PW's laboratory at Emory University for solid phase extraction and radioimmunoassay.

Every effort was made to collect samples at the same time each morning (0800–1200) to reduce bias that might be introduced if fT excretion exhibited the diurnal variation reported for sT (Van Horn et al., 1976). Broad diurnal variations in steroid excretion were assessed by analyzing temporal variation in the T content of 38 morning and 25 afternoon (1400–1800) fecal samples collected from four adult male sifaka.

#### Analysis of fecal samples to estimate fT

**Hormone assay.** Each male's dried fecal pellets from a single void were quantified using solid phase extraction techniques discussed previously in Brockman and Whitten (1996) and radioimmunoassay procedures described below. In the extraction procedure recovery of radiolabeled testosterone averaged 65%.

The testosterone radioimmunoassay procedures followed the microassay procedures of Beall et al. (1992) using reagents from the Equate Testosterone RIA kit (Binax, South

TABLE 1. *Social group composition*

	Birth year	Age (years)	Sex-specific rank	Group tenure (years)
Vaovao: 1990–91				
Females				
20	1970 (est)	22	1	7
19	1975 (est)	15	2	7
80	1985	5	3	5
Males				
240	1984 (est)	6	1	1
Fd	1986 (est)	4	2	1
Vavy Masiaka: 1991–92				
Females				
36	1976 (est)	15	Undecided	7
107	1980 (est)	11	Undecided	7
Males				
146	1980 (est)	11	1,2 (see text)	2
140	1982 (est)	9	2,1 (see text)	2
UC	1986 (est)	4	3	2.5 (see text)
228	1990	1	—	1
243	1983 (est)	8	1 (see text)	Immigrated 1/1992

Data according to Richard (unpublished).

Portland, ME). Working buffer was 0.1% gelatin phosphate buffered saline (pH 7.4). An aliquot of fecal extract was evaporated using nitrogen and reconstituted in buffer at a 1:50 dilution.  $^{125}\text{I}$  testosterone tracer (50  $\mu\text{l}$ ) and 100  $\mu\text{l}$  antiserum diluted 1:2 in working buffer were added to aliquots (100  $\mu\text{l}$ ) of standards (diluted 1:10 to give concentrations of 1–100 ng/dL), samples, and controls (diluted 1:10). Each was vortexed and incubated overnight at room temperature. The following morning, 500  $\mu\text{l}$  second antibody, diluted 1:2, was added, and the incubates were vortexed and centrifuged at 1500g for 60 minutes at 4°C. Following decanting of the supernatants, the radioactivity of the precipitate was determined by 10 minute counts in a Packard RIAsTAR gamma counter (Packard, Downer's Grove, IL) with Expert QC software. Cross-reactivities for the testosterone antibody were 1.7% for dihydrotestosterone, 0.08% for 5 $\alpha$ -androstane-3 $\alpha$ , 17 $\beta$ -diol, and less than 0.06% for epitestosterone, androstenedione, androstenedione, androsterone, dihydroepiandrosterone, epiandrosterone, epietiocholanolone, estradiol, and progesterone.

**High performance liquid chromatography (HPLC).** Chromatography was carried out using a Perkin-Elmer (Norwalk, CT) HPLC system and a Hypersil ODS column (5  $\mu\text{m}$ , 25 cm  $\times$  4.6 mm I.D., Aldrich, Milwaukee, WI). Androgens and related me-

tabolites were separated using an acetonitrile:water gradient of 60–80% in 12 minutes, 80–100% in 5 minutes with a flow rate of 1 ml/minute at 45°C and UV detection at 280 nm and 240 nm. Aliquots (20  $\mu\text{l}$ ) of fecal extracts were injected in mobile phase, and fractions of the chromatographic eluent were collected at 0.3 minutes or from 0–17 minutes and assayed for testosterone immunoreactivity. One-fifth (20  $\mu\text{l}$ ) of the 100  $\mu\text{l}$  aliquot used in RIA was injected in mobile phase and fractions were assayed at dilutions of 1:1, 1:1.5, and 1:10 to yield overall dilutions of 1:5, 1:8, and 1:50.

#### Assessment of hormone-behavior relationships

Hormone-behavior relationships were assessed using correlations between fT levels and frequencies of behavior. Our rationale for employing this, rather than a predictive, approach is based upon previous studies of captive primates showing that hormone-behavior interactions have not always demonstrated a one-to-one relationship between T and rank, sexual behavior, or aggression. However, these studies have shown that T is most consistently related to aggression, although it can be modified by social context (Whitten, in press). This variability therefore makes it difficult to test the predictions of fT as an index of behavioral response in a linear fashion.

## RESULTS

### Steroid recovery and radioimmunoassay validity

Serial dilutions of a fecal extract from a sifaka male provided evidence of parallelism. The curve of expected dose vs. observed percent bound obtained from serial dilutions of fecal extract ( $\text{logit } \% \text{ bound} = -1.47 \log \text{ dose} + 1.74$ ,  $n = 6$ ,  $r = 0.999$ ) paralleled the standard curve ( $\text{logit } \% \text{ bound} = -1.42 \log \text{ dose} + 1.65$ ,  $n = 6$ ,  $r = 0.999$ ). The statistical significance of the observed parallelism was tested using constrained curve fitting with a logistic curve fitting procedure (DeLean et al., 1978). Constraining the curves to share a common slope did not significantly alter the goodness of fit ( $F = 1.06$ ,  $P = 0.35$ ), indicating that the dose-response curves were parallel. Tests of accuracy indicated excess recovery ( $129 \pm 9\%$  over a range of 1–16 ng/ml) of unlabeled T added to a fecal extract diluted 1:10 (observed = 1.22 expected + 2.3), suggesting interference by the fecal matrix. The intraassay coefficient of variation of a fecal pool was 3.4% ( $n = 6$ ). The interassay coefficient of variation was 4.6% ( $n = 4$ ) for a low serum control and 2.2% ( $n = 4$ ) for a high serum control.

Preliminary HPLC analyses indicated that metabolites other than T contributed to the observed immunoreactivity when samples were assayed undiluted. Peaks corresponding to the retention time of epietiocholanolone and epiandrosterone accounted for 25% and 8% of the immunoreactivity, a pattern of cross-reactivity similar to that seen in human stool samples (Sobolik et al., 1996). In addition, an unidentified chromatographic peak at 6.3 minutes, just after the retention time for T (6.0 minutes) also appeared to contribute to immunoreactivity. The addition of T to an aliquot of the extract showed that this peak was distinct from T. Chromatography of additional samples indicated that the unidentified peak was only in a few of the samples with higher T concentrations. Figure 1 shows the distribution of immunoreactivity in fractions collected from samples with (M140) and without (M243) the unknown peak. Fractions were further diluted in an attempt to reduce the contribution of metabolites. A dilution equivalent to a 1:50

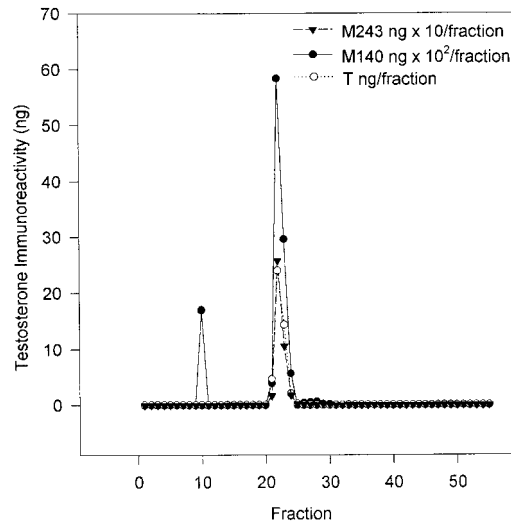


Fig. 1. Immunoreactivity of HPLC fractions of fecal extracts from male *Propithecus verreauxi*.

dilution of extract substantially reduced the contribution of the other metabolites. Figure 1 shows that only a single major immunoreactive peak, corresponding to the retention time of T and representing 84–99% of the immunoreactivity, could be detected in the HPLC-separated fractions regardless of whether the unidentified metabolite was present or not. For example, an aliquot of M140's fecal extract estimated by RIA to contain 1.25 ng yielded a total immunoreactivity of 1.16 ng in chromatographic fractions. Of this total, 0.974 ng (84%) of the immunoreactivity occurred in fractions 21–24, coincident with the T standard (fractions 21–24). An additional 0.17 ng (15%) eluted in fraction 10 (a pattern also seen in human stool samples: Sobolik et al., 1996) and 0.02 ng (1.7%) eluted in fractions 26–30. In M243's sample, 99% of the immunoreactivity occurred in the T elution zone (fractions 21–24). These results indicated that the 1:50 dilution resulted in an accurate estimate of T concentration, and this concentration was used in subsequent assays of male samples.

### Diurnal variability in steroid concentration

The influence of time of fecal collection on steroid concentration was examined in morning and afternoon samples obtained from four adult males across a 4 month period.



Morning and afternoon fecal samples had median T concentrations of  $96.0 \pm 61.7$  (SEM) ng/g and  $67.5 \pm 124$  (SEM) ng/g respectively, suggesting no significant time effect of collection (Mann-Whitney rank sum test:  $T = 795$ ,  $P = 0.950$ ).

#### Mating and its demographic context

Vaovao and VM group members were observed for a total of 790 hours over the 3 (VV) and 3.5 month (VM) field seasons. Mating occurred January 9–21, 1991, and February 10–23, 1992, in the VV and VM groups, respectively. Of the three females who exhibited hormonal evidence of conception (Brockman, 1994; Brockman and Whitten, 1996) only VM female 36 produced an offspring the following birth season (Richard, unpub. data). The fate of VV80's conception is unknown, but VV20 exhibited hormonal evidence of abortion 54 days post-conception, associated with increased levels of intergroup encounters (Brockman, 1994; Brockman and Whitten, 1996).

Copulations occurred under markedly different demographic circumstances in the two study groups. In contrast to VV whose composition remained stable during the breeding season, VM experienced a shift in male membership in mid-January 1991 after an intensely aggressive encounter between older resident males 146 and 140. During 2 days of bloody fighting an unmarked adult male (captured and marked 243 in 1993) immigrated into VM peripheralizing males 146 and 140. The presence of this new resident male precipitated male UC's transfer into a neighboring group 2 weeks later, the consequence of continued aggression from the former.

#### Levels and temporal variation of testosterone excretion

While there was no significant seasonal effect on weekly mean levels of fT of adult males between the August portion of the birth season and the January–March breeding season (birth season mean:  $182.7 \pm 254.0$  (SD) ng/g; breeding season mean:  $262.3 \pm 213.3$  (SD) ng/g; Mann-Whitney rank sum test:  $T = 17$ ,  $P = 0.35$ ; Fig. 2), this may be because three of the six males sampled in August had markedly elevated fT levels on

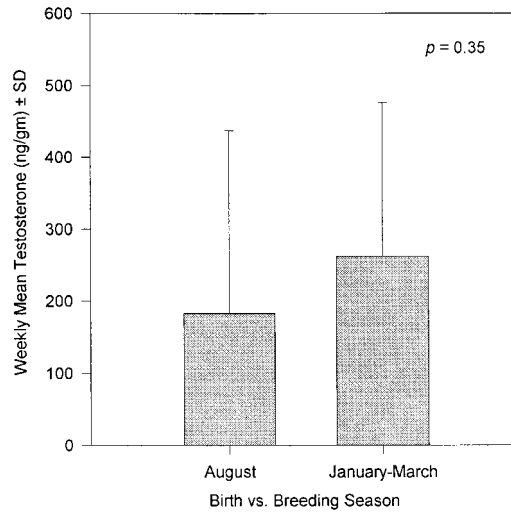


Fig. 2. Birth season ( $N = 6$  males) vs. breeding season ( $N = 6$  males) levels of weekly mean fecal testosterone in adult male *Propithecus verreauxi*.

days in which group reorganization, male transfer, and aggression occurred. Removing these values from the analysis resulted in significant seasonal effects on T excretion (birth season mean:  $41.1 \pm 25.9$  (SD) ng/g; breeding season mean:  $262.3 \pm 213.3$  (SD) ng/g; Mann-Whitney rank sum test:  $T = 8$ ,  $P = 0.025$ ).

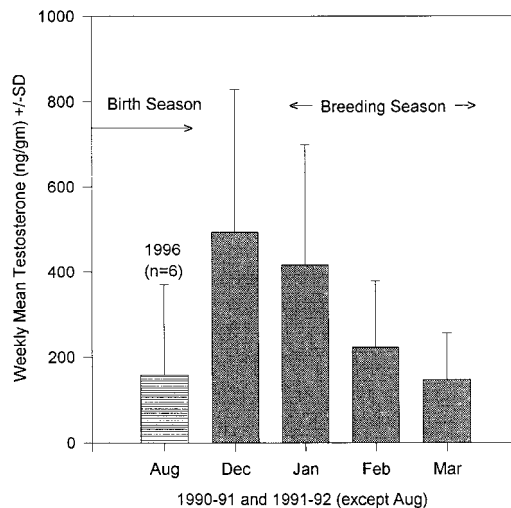


Fig. 3. Weekly variation in mean testosterone concentrations excreted by adult male *Propithecus verreauxi* during the August portion of the birth season vs. January–March breeding season.

Breeding season fT levels of sexually mature ( $n = 6$ ) and immature ( $n = 1$ ) male sifaka averaged 249.1 ng/g and 34.6 ng/g, respectively. Fecal T of adult males did not vary significantly across the 4 month sampling period (repeated measures ANOVA:  $F = 1.34$ ,  $P = 0.322$ ), but March levels were substantially lower than those of the previous 3 months, although not significantly so ( $t$ -test:  $t = 2.48$ ,  $P = 0.056$ ; Fig. 3). The March decline in fT followed mating and conception in both groups, and in fact, post-conception fT averages were significantly lower than preconception averages (preconception mean:  $346.9 \pm 224.8$  (SD) ng/g; post-conception mean:  $107.7 \pm 77.5$  (SD) ng/g; paired  $t$ -test:  $t = 2.782$ ,  $P = 0.05$ ) but remained higher in the two alpha males than in their subordinates ( $t = 4.145$ ,  $P = 0.026$ ). Vavy Masiaka, but not VV, males exhibited significantly elevated fT levels during a 2–3 week period between mid-December and mid-January (Mann-Whitney rank sum test: VM:  $T = 48$ ,  $P = 0.01$ ; VV:  $T = 25$ ,  $P = 0.083$ ), associated with extragroup matings, intergroup and intragroup aggression, and group reorganization.

#### Male age and rank

Weekly fT levels varied significantly across males (repeated measures ANOVA:  $F = 5.06$ ,  $P < 0.001$ ; Table 2), fT levels of adult males being significantly higher than those observed in subadults and juveniles (Mann-Whitney rank sum test:  $T = 425$ ,  $P < 0.0001$ ; Table 3, Fig. 4).

Among mature males, fT was significantly higher in dominants than in subordinates during the 13 weeks of the study when rank was stable in both social groups (dominant mean:  $338.8 \pm 287.4$  (SD) ng/g; subordinate mean:  $65.2 \pm 22.7$  (SD) ng/g; paired  $t$ -test:  $t = 3.49$ ,  $P = 0.004$ , Fig. 5), but it did not differ significantly by rank during the mid-December to mid-January period of elevated fT ( $t = -1.08$ ,  $P = 0.359$ ).

Although high ranked males had higher fT levels than subordinates, levels varied weekly by study group. In VV, dominant M240's fT levels were significantly higher than those of the subordinate, subadult MFd throughout the sample period (Mann-Whitney rank sum test:  $T = 112.5$ ,  $P = 0.017$ ,

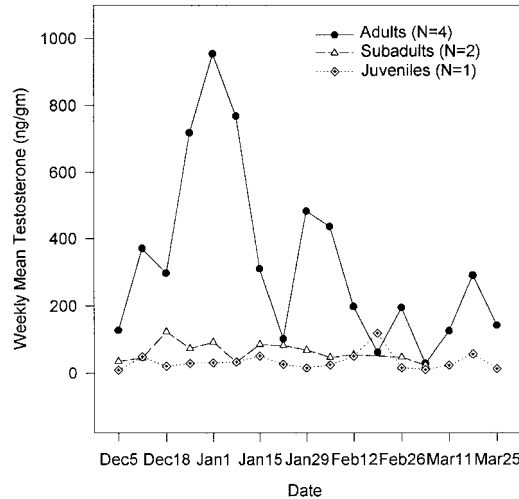


Fig. 4. Age-specific weekly variation in mean fecal testosterone levels in male *Propithecus verreauxi* during the breeding season ( $N = 7$  males).

Table 2, Fig. 6). In VM, dominant M146's fT levels were higher than those of the subordinate M140 in 2 of the first 3 weeks of December but fell below those of M140 in the mid-December to mid-January sampling period (Fig. 7). Male 146's fT levels remained significantly lower thereafter (Wilcoxon signed rank test:  $W = 66$ ,  $P = 0.001$ ) following a dominance reversal and subsequent peripheralization of both males due to the immigration of a new male, M243, January 12–13. After immigration, M243 retained significantly higher fT levels than those of the two peripheralized and subordinate males 146 and 140 throughout the rest of the sampling period (paired  $t$ -test:  $t = 2.972$ ,  $P = 0.016$ ).

#### Female cycles and sexual activity

Weekly variation in fT did not appear to be closely related to sexual activity (Fig. 8). Fecal T was not significantly higher during weeks in which a follicular phase (Mann-Whitney rank sum test: VV:  $T = 11$ ,  $P = 0.523$ ; VM:  $T = 57$ ,  $P = 0.802$ ), an estradiol peak (VV:  $T = 51$ ,  $P > 0.1$ ; VM:  $T = 58$ ,  $P > 0.1$ ), or within-group mounts or copulation occurred (VV:  $T = 111$ ,  $P = 0.523$ ; VM:  $T = 70$ ,  $P > 0.1$ ). Nor were the fT concentrations of individual males significantly higher during weeks in which they were observed

TABLE 2. Inter-individual differences in absolute levels of fecal testosterone among male *Propithecus verreauxi*

	Age (years)	Mean (SD) (ng/g)	Median (SEM) (ng/g)	Max (ng/g)	Min (ng/g)	N <sup>1</sup>
Vaovao: 1990–91						
240	6	298.3 ± 338.5	123.8 ± 90.47*	973.5	18.00	14
Fd	4	69.0 ± 43.8	51.8 ± 12.64	171.0	24.00	13
Vavy Masiaka: 1991–92						
243	7	543.3 ± 414.4	495.0 ± 124.94**	1535.0	67.50	11
140	10	416.1 ± 737.7	94.8 ± 184.41**	2250.0	24.00	16
146	12	116.4 ± 142.6	61.5 ± 35.65**	531.0	24.00	16
UC	4	66.4 ± 24.4	60.0 ± 8.14**	104.3	36.00	9
228	1–2	34.6 ± 26.4	27.0 ± 6.40**	118.0	9.30	17

<sup>1</sup>Number of fecal samples.\**P* = 0.01.\*\**P* = 0.05.

to mount or copulate (paired *t*-test: *t* = −0.777, *P* = 0.480).

However, in VM (Fig. 7), M243 exhibited mating-related fT elevations during F107's estradiol peak and, prior to their peripheralization, both males 140 and 146 exhibited fT elevations during the weeks when extra-group matings occurred [which may have coincided with estradiol elevations in the neighboring group since almost all mating was associated with estradiol peaks (Brockman and Whitten, 1996), Fig. 7]. However, these elevations also may reflect intragroup and intergroup agonism (see below).

#### Intragroup aggression

Although weekly variation in fT levels in adult males was not significantly associated with intragroup aggression rates in either social group (VV: *r*<sub>s</sub> = 0.28, *P* = 0.324; VM: *r*<sub>s</sub> = 0.339, *P* = 0.179, Fig. 9), some males exhibited higher fT levels during within-group aggression than other males. Male-male aggression was uncommon in VV and was observed only during the 3 week period of mating activity (Fig. 6), associated in MFd's case, with intense guarding by M240

and copulatory harassment by M240 and F19.

In VM, the mid-December to mid-January fT elevations in males 146 and 140 preceded their rank reversal and were more marked in M140 (Fig. 7) in spite of the fact that M146 was still unambiguously dominant, initiating and winning all aggressive interactions with M140 prior to and including the bloody fight on January 12. The continuation of this fierce fight on January 13 culminated in a dominance reversal and the immigration of M243. Male 140 was driving the continuously "sifaking" M146 from the group when M243 entered VM, assuming the position of dominant resident male. Male 140's

TABLE 3. Age-specific differences in fecal testosterone levels in male *Propithecus verreauxi*

	Mean (SD) (ng/g)	Median (SEM) (ng/g)	N <sup>1</sup>	N <sup>2</sup>	N <sup>3</sup>
Age class					
Juvenile	34.6 ± 26.40	27.0 ± 6.40	1	17	17
Subadult	62.9 ± 27.90	55.5 ± 7.73	2	14	21
Adult	330.3 ± 266.90	291.8 ± 64.73*	4	17	57

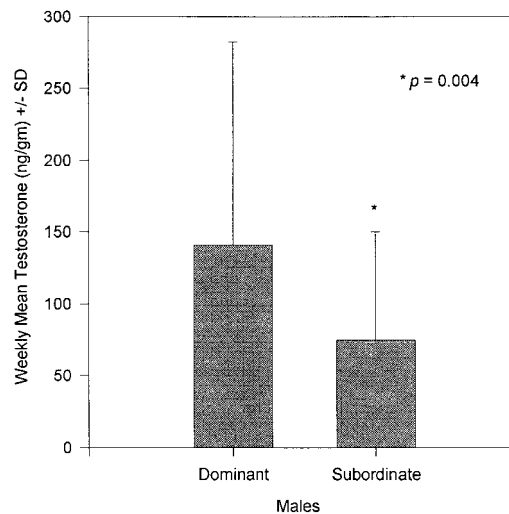
<sup>1</sup>Number of males sampled.<sup>2</sup>Number of weeks sampled.<sup>3</sup>Number of fecal samples.\**P* < 0.0001 vs. juvenile and subadult males.

Fig. 5. Rank-related levels of fecal testosterone in adult male *Propithecus verreauxi* under stable conditions.



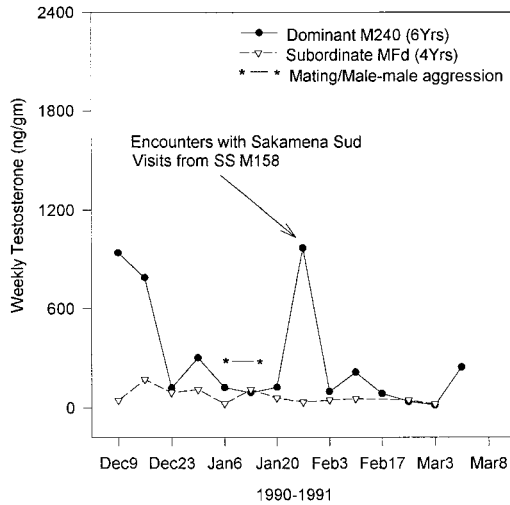


Fig. 6. Temporal variation in weekly fecal testosterone levels in Vaovao males associated with rank, mating, and aggression.

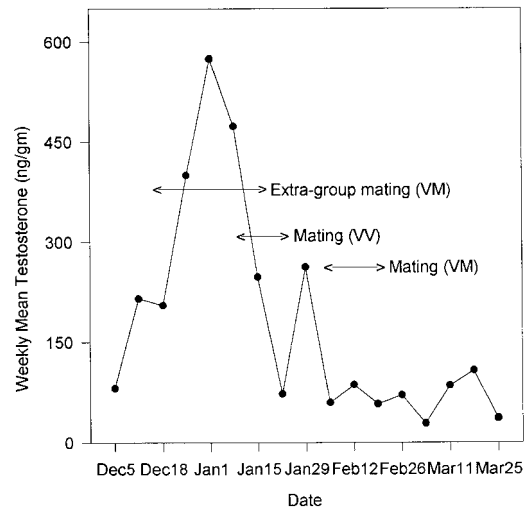


Fig. 8. Composite profile of estrus-related weekly mean fecal testosterone levels in sexually mature male *Propithecus verreauxi*.

subsequent attempts to rejoin and, with MUC's aid, expel the intruder, were unsuccessful; both M140 and M146 became peripheral group members, restricted to following and occasionally approaching VM residents. Although both males 140 and 146 experienced significant and sustained reductions in weekly fT levels after M243's immigration (combined weekly mean fT levels before

M243:  $535.9 \pm 560.3$  (SD) ng/g; after M243:  $59 \pm 22.5$  (SD) ng/g; Mann-Whitney rank sum test:  $T = 91$ ,  $P < 0.007$ ), the reduced levels appeared to be sufficient to support sexual behavior since both males were observed mating with resident F36 on the periphery of the social group February 10 (Fig. 7). Affiliations with VM members terminated 6 months later, however, when males 140 and 146 were observed roaming together as solitary males; they were not seen again in subsequent annual censuses (Richard, unpub. data).

The new alpha male, M243, exhibited the highest levels of and greatest temporal variation in fT (Table 2, Fig. 7). Elevations in mid-January and early and late-February were associated with mating with resident F107, interactions being marked by relentless attacks by the female whenever M243 approached. Although fiercely resistant to M243's mating attempts most of the day, F107 immediately ceased all hostilities when the male was able to secure and maintain flank contact via a mount. She subsequently presented and mated with M243 three times; endocrine data suggested, however, that the cycle was anovulatory (Brockman, 1994; Brockman and Whitten, 1996).

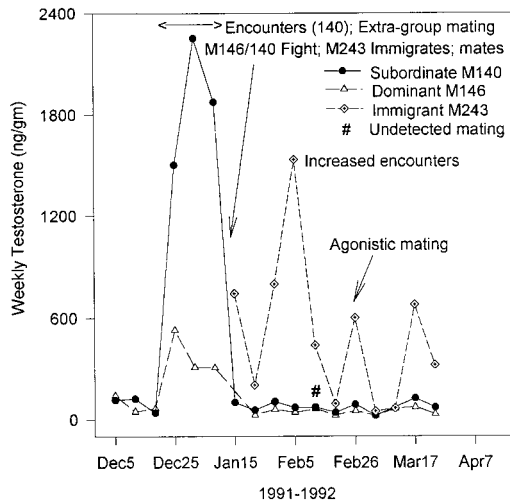


Fig. 7. Temporal variation in weekly fecal testosterone levels in Vavy Masiaka adult males associated with rank, mating, and aggression.

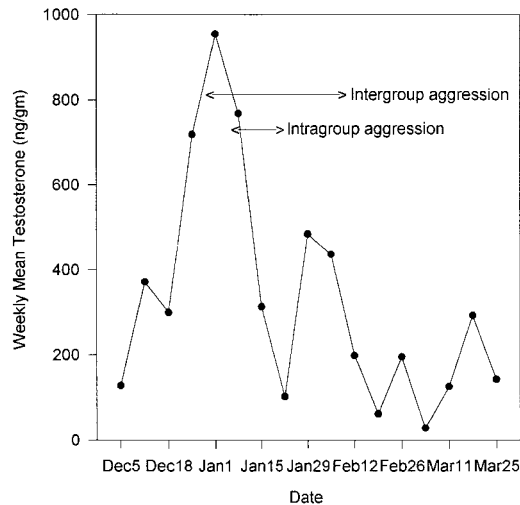


Fig. 9. Composite profile of aggression-related weekly mean fecal testosterone levels in adult male *Propithecus verreauxi*.

### Intergroup aggression

Weekly average fT levels in adult males were significantly and positively associated with intergroup encounter rates in VM but not in VV (VM:  $r_s = 0.50$ ,  $P = 0.04$ ; VV:  $r_s = -0.20$ ,  $P = 0.49$ ), most likely because intergroup encounters were infrequent among VV males. Only 11 of the 35 VV encounters with neighboring groups involved resident males, and these appeared to consist primarily of backing up the resident females in their aggression with neighboring females. In marked contrast, VM males participated vigorously in intergroup aggression and were the sole participants in 27 of the group's 32 encounters with neighboring groups. Intergroup aggression, in the context of sifaka reproductive behavior, typically involves high speed arboreal chases, characterized by stares, growls, rapid reciprocal rushes, and scent marking, but rarely fights (Jolly, 1966; Richard, 1992). In this study, only three of the 67 encounters involved fierce physical combat.

Intergroup encounters coincided with elevations in fT in both social groups (Fig. 9), but with marked intergroup variations in magnitude and duration of T excreted. In VV, M240's February 27 elevations in fT coincided with an escalation in aggressive

encounters with the neighboring Sakamena Sud group and intermittent visits from its resident M158 (Fig. 6). Although interactions between M158 and the VV males were predominately affiliative (only 14% involved chasing whereas 86% involved greeting, grooming, and playing), M158 was a potential intruder since he had been a VV resident prior to the immigration of males 240 and Fd the previous year. Richard et al.'s (1993) study of male dispersal shows that male transfers are often preceded by periods of wandering and visiting and that, occasionally, subadult males return to groups they have previously occupied (Richard, unpub. data).

Among adult VM males, 140 and 243 exhibited significant correlations between fT levels and intergroup aggression rates (M140:  $r = 0.86$ ,  $P = 0.03$ ; M243:  $r = 0.68$ ,  $P = 0.03$ ; M146:  $r = 0.57$ ,  $P = 0.24$ ). The fact that M140 appeared to exhibit a stronger androgen response to intergroup aggression than M146 (Fig. 7) may be explained by his 3:1 higher rate of participation in these encounters. Following his transfer into VM, M243 engaged in 21 intergroup encounters, 12 associated with the mid-January and early February fT peaks. Although the January fT elevations observed for males 140, 146, and 243 coincided with extragroup matings (January 7, 10, 16), it is unclear to what degree, if any, these copulations may have augmented fT concentrations. Subadult MUC and juvenile M228 also exhibited elevations in fT (Fig. 10) associated with intramale aggression with immigrant M243 in the former and visits by extragroup males 158 and UC in the latter.

## DISCUSSION

### Radioimmunoassay of fT

These investigations show that radioimmunoassay methods reliably assess T in fecal extracts from male *P. verreauxi* and, when combined with behavioral data, provide insights into the seasonal, developmental, and social factors mediating reproductive behavior in free-ranging populations. Validation experiments indicated that the T radioimmunoassay displayed parallelism and predictable recovery in sifaka fecal extracts, but provided evidence of interference

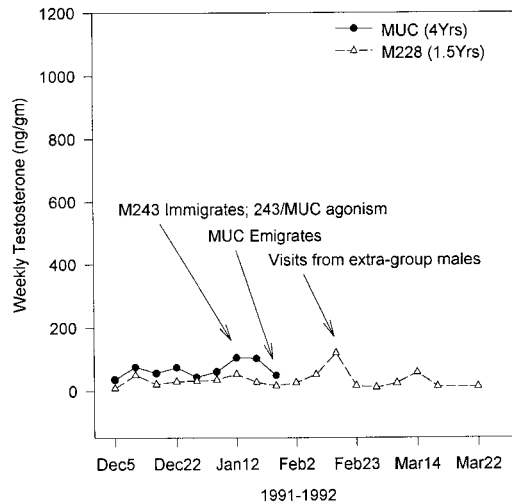


Fig. 10. Temporal variation in weekly fecal testosterone in Vavy Masiaka subadult and juvenile males.

of the fecal matrix with the second antibody. The HPLC analyses indicated that the estimated doses represented authentic T.

#### Hormone-behavior relationships

The context-dependent nature of T secretion (Whitten, in press) made it difficult to define a single behavioral measure that could be used to test the validity of fT as an index of hormone-behavior relationships. Therefore, fT profiles were compared to a variety of behavioral measures (rank, intra-group aggression, intergroup aggression, and sexual behavior), as well as maturational and seasonal stages, that have been reported to reflect sT. The association of changes in fT with breeding season events (i.e. conception), developmental stages, and intergroup aggression suggests that fT did reflect important behavioral and physiologic events.

#### Seasonal patterning of T excretion

The unremarkable seasonal variation in fT levels observed in this study contrasts with the marked seasonality in captive male strepsirrhines where increased androgen activity is signaled by larger testes (Petter-Rousseaux, 1970; Bogart et al., 1977a; Perry et al., 1992) and higher sT levels (Evans and Goy, 1968; Bogart et al., 1977b) during the

breeding season. The fact that seasonal effects become significant in sifaka when birth season fT levels from migrating males are removed from the analysis suggests that aggression-related increases in fT may dampen seasonal variations of androgen levels in free-ranging populations. The breeding season pattern of T excretion is consistent with previous studies of breeding season variations in fecal steroids in free-ranging females (Brockman, 1994; Brockman and Whitten, 1996); T elevations spanned a minimum 4 month period, but whereas ovarian steroid elevations coincided with the January onset of the breeding season, fT peaked 1 month earlier, gradually declining thereafter to a nadir in March. Reductions in fT at the end of the breeding season were less marked in dominant males and may reflect the prolongation of testicular activity.

#### Developmental and social factors affecting T excretion

The patterning of individual differences in fT also resembles patterns reported for sT, where developmental stage as well as rank, aggression, and intergroup interactions may influence circulating concentrations (reviewed in Izard, 1990). The consistently diminished levels of fT in subadult males (i.e. Fd and UC) could result from a number of factors including kin-related developmental inhibition and low rank. In Foerg's (1982) study of *Varecia variegata*, testes of 3-year-old males that remained in their natal group during the breeding season remained small, but when the males were paired with unrelated adult females in the absence of adult male kin, testes size was comparable to that of adult males. In Perret's (1977) study of adult male *Microcebus murinus*, subordinate males exhibited significantly lower sT concentrations than dominants. The fact that males UC and Fd were not natal males in these social groups (Richard, unpub. data) suggests that rank may be a stronger explanation for diminished fT levels in subadult sifaka than kin-based inhibition. Albeit low, fT concentrations were nevertheless sufficient to support reproductive behavior in subadults. Although VM male UC was never observed competing for copulations, he obtained a brief mount in late December when

fT levels were low. This low level of sexual behavior contrasts markedly with that shown by VV's subadult MFd that was relentless in his pursuit of estrous females. The reasons for this are unknown, but one explanation may be the demographic composition of the social groups and the potential costs associated with increased intrasexual competition in VM; the presence of more adult males effectively guarding fewer estrus females. Alternatively, variations in subadult male sexual behavior may have been a consequence of differences in food availability at these two sites. This is unlikely however, because the vegetative characteristics of the groups' home ranges were similar, composed of a continuous north-south riverine forest bordering the Sakamena River.

Variance in fT among adult males appeared to be influenced by rank dynamics and intermale aggression. The immigration and rise to dominant status of M243 was associated with the fall of fT in males 140 and 146 and the maintenance of persistently higher fT in M243. Insufficient data are available to determine if the dominant status of M146 was similarly associated with persistently elevated fT, but it is clear that relative fT levels were reversed in males 140 and 146 in the 1 month period prior to their reversal in rank.

Dominance has been associated with higher sT only during periods of rank instability in macaques and baboons (*M. fuscata*: Eaton and Resko, 1974; *M. mulatta*: Gordon et al., 1976; *P. anubis*: Sapolsky, 1982, 1986), but may be a more persistent attribute of high rank in species such as mandrills (Wickings and Dixon, 1992) and guenons (Eberhardt et al., 1980; Steklis et al., 1985) where the alpha position is markedly superior to subordinate ranks. Further investigations will be required to assess which pattern is characteristic of sifaka. The entire breeding season may have represented a period of rank instability in VM, reflecting events leading to the reversal of rank in males 140 and 146, their loss of rank and peripheralization, and M243's subsequent attempts to consolidate his position with the resident females. However, rank instability does not

explain the rank-related patterning of fT in VV.

Regardless of which of these models characterize sifaka, it is likely that the observed pattern of T excretion in sifaka will reflect the patterning of aggression as it does in catarrhine primates, where T is generally more closely related to aggression than to rank (Whitten, in press). In this study, declines in fT were associated with intragroup aggression whereas elevations in fT were more clearly associated with intergroup aggression than with sexual activity or intragroup aggression. Previous studies indicate that while exercise and aggression elevate T levels in male primates, including humans, these increases predominantly occur in winners of contests, losers tending to exhibit suppressed T concentrations (Rose et al., 1972, 1975; Bernstein et al., 1974 in Sapolsky, 1993). Although M140 was clearly the loser in his agonistic interactions with M146 prior to January 12, these suppressive effects may have been offset by his significantly higher level of participation in intergroup aggression. Similar modulating androgen-behavior interactions are reported to occur in subordinate primates wherein aggression-related T elevations are offset by the inhibitory stress effects of injury and defeat (Sapolsky, 1993). Alternatively, the androgen-behavior disjunction observed in M140 may indicate that his dominance relationship with M146 was already changing, the androgen levels perhaps indicating rank-related responses not yet evident in behavior. Although the precise nature of these interactions is unclear in sifaka, the fact remains that physical combat between males 140 and 146 was extremely costly. Not only did these males sustain injuries substantial enough to compromise their ability to ward off the immigration of a new male, but their resultant peripheral status led ultimately to the loss of group membership and an uncertain future.

Increased aggression may also have been implicated in the fT elevations observed in VM males 243 and UC, associated with, in the case of M243, agonistic mating with resident females and group reorganization. With the exception of M243, male sifaka exhibited low fT concentrations during

within-group mating activity. One explanation for M243's androgen response may be that this "agonistic mating" elevation in fT is less indicative of mating success than it is of aggression. Support for this hypothesis comes from Sapolsky's (1982) studies of free-ranging *P. anubis* wherein high sT titers were reported to be associated with male aggressiveness, but not copulatory success.

In VM, mating appeared to be influenced by the availability of extragroup females and female mate choice. All three VM adult males mated with neighboring VV females during the mid-December to mid-January period of increased intergroup aggression. Male 243 mated with F80 during an encounter 3 days after immigrating into VM while males 140 and 146 copulated with F20 during the early January elevation in fT coincident with visits to, and encounters with, the VV social group. Subsequent to their peripheralization, these males also mated with resident F36 when she solicited copulations with them on the periphery of the social group. Although demographic data (Richard, unpub. data) showed that these females gave birth the following birth season, paternity data indicating identity of the fathers are lacking. This caveat notwithstanding, these results suggest that male reproductive fitness may be enhanced through increased interactions with neighboring groups and female preferences for certain familiar males (Brockman, 1994; Brockman and Whitten, 1996). The fact that extragroup mating coincided with increased intermale aggression in VM, but not VV, males suggests that there may be important individual and group-related differences in male mating strategies.

Finally, field studies of catarrhine primates (Sapolsky, 1983) suggest that group reorganization can have a negative effect on sT concentrations in group males subsequent to social disruption, associated with stress effects on testicular function. Hormonal data from males 146 and 140 before and after M243's immigration into VM provide the most credible evidence for such a potential relationship in sifaka. For males 146 and 140 combined, fT concentrations decreased 79% and remained diminished after M243's immigration into the social

group, the latter, we would argue, indicative of the stress effects of social peripheralization on fT concentrations.

The small number of subjects and samples examined in this study preclude any definitive conclusions about androgen-behavior relationships in sifaka. Moreover, until experimental evidence is obtained that fT actually reflects gonadal activity or sT, the results of these investigations should be accepted with caution. These caveats notwithstanding, we believe that the behavioral data provide clues to some of the social factors affecting androgen activity in adult male sifaka including, but not limited to, intergroup aggression and group reorganization. This study also suggests that resident males that engage in intense male-male aggression during the breeding season may incur high costs, including reduced fitness, group expulsion, and perhaps, lowered survivorship. This is consonant with previous studies of free-ranging passerine birds (Duffy, 1989) showing that reproductive and survivorship costs result from prolonged high levels of sT during aggression. While this androgen-aggression relationship is not yet firmly established in sifaka, if the hypothesized costs of increased aggressiveness in males are borne out in future studies, then these results may provide new insights into the social constraints affecting the evolution of male body size in this and other monomorphic primates.

In conclusion, the results of this study suggest that 1) solid phase extraction and radioimmunoassay techniques are reliable and accurate methods for quantifying T in sifaka feces; 2) fecal T elevations span a minimum 4 month period, peak levels occurring the month preceding the January onset of the breeding season; 3) fecal T concentrations are influenced by developmental factors and, among mature males, social factors associated with rank, intergroup aggression, and group instability.

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